# CLINICAL STUDIES WITH GONADOTROPIN-RELEASING HORMONE*

RAPHAEL JEWELEWICZ, M.D.

Assistant Attending of Obstetrics and Gynecology
Columbia-Presbyterian Hospital
Assistant Professor

INGE DYRENFURTH, Ph.D.

Assistant Professor

MICHELLE WARREN, M.D.

Assistant Professor

RAYMOND L. VANDE WIELE, M.D.

Director, International Institute for the Study of Human Reproduction
Professor and Chairman
Department of Obstetrics and Gynecology
Columbia University
New York, N. Y.

THE existence of hypothalamic substances which regulate the function of the anterior pituitary was postulated by Harris about 40 years ago.[1] However, only in the last few years has it been possible to prove his hypothesis. The isolation, structural determination and, finally, synthesis of several of the releasing hormones in the hypothalamus has been accomplished only recently. These hormones are now available for clinical study and their diagnostic and therapeutic uses are being evaluated.

## HISTORICAL BACKGROUND

About 35 years ago, when the pituitary was still "the master gland," several physiologists asked: What controls pituitary secretion? It was known that the hormones secreted by the posterior pituitary, oxytocin and vasopressin, were also found in the hypothalamus. Later it was

*Presented before the Section on Obstetrics and Gynecology of the New York Academy of Medicine March 13, 1974.

This investigation was supported in part by U.S. Public Health Service Research Grant 5P01 HD 05077 from the National Institutes of Child Health and Human Development, Bethesda, Md.

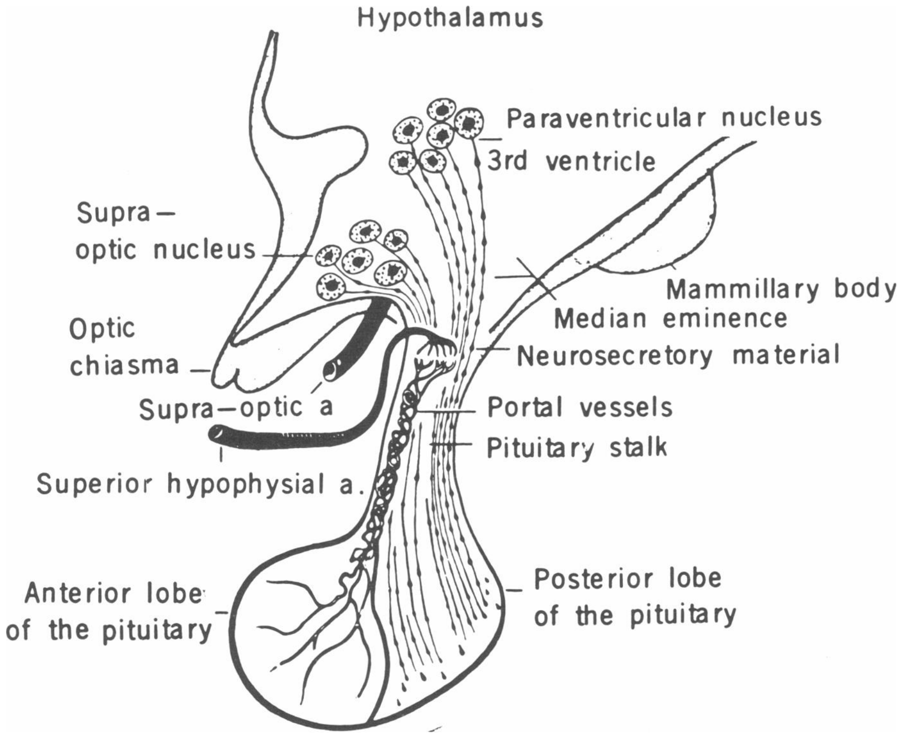


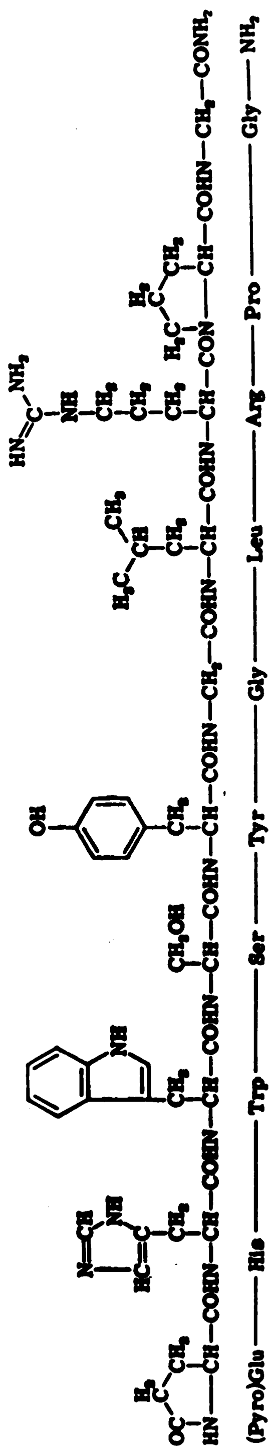

Fig. 1. Schematic representation of the anatomical interrelation between the hypothalamus and the pituitary.

shown that these two hormones are actually manufactured in specialized nerve cells in the hypothalamus. They then flow down the pituitary stalk through the axons of hypothalamic nerve cells, and are stored in the posterior pituitary, from which they may be secreted into the bloodstream after a proper physiological stimulus has been delivered. This observation led Drs. E. and B. Scharrer to formulate the new concept of neurosecretion.[2] They suggested that specialized nerve cells might be able to manufacture and secrete true hormones, which would then be carried by the blood to exert their effects in target tissues or organs remote from their point of origin. While these concepts were being formulated, it was found that the anterior pituitary behaved differently. Indeed it was already known that the anterior and posterior pituitary originate from different structures. Although there are no axons connecting the hypothalamus to the anterior pituitary, the function of the anterior pituitary is dependent on the integrity of the hypothalamo-pituitary structures.[3] In 1936 the pituitary portal system was discovered.[4] This is a bundle of tiny blood vessels which extend from the floor of the hypothalamus through the pituitary stalk to the anterior pituitary. In 1937 Harris proposed that hypothalamic control of the anterior pituitary could be neurochemical.[1] Substances manufactured by nerve cells in the hypothalamus could be released into the capillaries that run from the hypothalamus to the anterior pituitary, where they could stimulate specific cells to secrete certain hormones. This hypothesis—that pituitary function is controlled by neurohormones originating in the hypothalamus—was soon proved true on the basis of physiological studies in many animals. However, it took more than 30 years to isolate and characterize the postulated hypothalamic hormones.

## Anatomy

The hypothalamus (Figure 1) is an area of the diencephalon lying at the base of the brain, ventral to the thalamus, and forming the floor and part of the lateral wall of the third ventricle. It is bounded anteriorly by the optic chiasma and posteriorly by the mammillary bodies. The median eminence of the tuber cinereum, which is an expansion of the floor of the third ventricle, is connected to the pituitary by the pituitary stalk. In the posterior pituitary there are axons which come down from the hypothalamus. In the anterior pituitary there is a portal system of blood vessels between the median eminence and the pituitary. Hypothalamic nerve fibers of different types secrete chemical substances from their nerve endings into the capillaries in the median eminence. These substances are carried by the portal system to the pituitary gland, where they stimulate or inhibit the release of various anterior pituitary hormones.

The existence of at least nine hypothalamic hormones of the anterior pituitary is now fairly well established (see accompanying table).[5] Several of these substances affect synthesis as well as release of the pituitary hormones; in addition, some have inhibitory effects. For at least three pituitary hormones (prolactin, growth hormone [GH], and melanocyte-stimulating hormone [MSH]) there is a dual system of hypothalamic control—one system stimulates and the other inhibits release. The need for an inhibitory system for these hormones can be



Molecular structure of LH- and FSH-releasing hormone (LH-RH/FSH-RH).

Fig. 2. Chemical structure of gonadotropin-releasing hormone (GnRH).

Hypothalamic Hormones Known to Control the Release of Pituitary Hormones

| |
|---|
| Corticotropin-releasing hormone |
| Thyrotropin-releasing hormone |
| Luteinizing hormone/releasing hormone* |
| Follicle stimulating hormone/releasing hormone* |
| Growth hormone/releasing hormone |
| Growth hormone/release-inhibiting hormone |
| Prolactin-releasing hormone |
| Prolactin release-inhibiting hormone |
| Melanocyte-stimulating hormone/releasing hormone |
| Melanocyte-stimulating hormone/release-inhibiting hormone |

*Gonadotropin-releasing hormone (GnRH): one hormone releases both LH and FSH.

explained by the absence of a negative feedback mechanism from the target organs, whereas the hormones secreted by the thyroid, adrenals, and gonads act upon the hypothalamus and pituitary and thus are able to control their own rates of secretion.[6]

## Luteinizing Hormone and Follicle-Stimulating Hormone/Releasing Hormone (LH-FSH/RH)

In 1971 Schally et al. reported the isolation, structural determination, and synthesis of a hypothalamic hormone which was able to stimulate secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in many laboratory animals and in human beings.[7] This substance was a relatively simple decapeptide (Figure 2). Soon after the chemical structure was revealed, it was synthesized, and large quantities became available for physiological and clinical studies. It has been shown that the effect of the synthetic and the natural LH-FSH/RH are the same.[7] When LH-FSH/RH is injected into animals or humans there is always a release of both LH and FSH. In view of these facts, Schally suggested that there is only one releasing hormone for both gonadotropins.[8, 9] However, this is still an unsettled problem. It is true that when LH-FSH/RH, whether natural or synthetic, is injected into animals or humans both LH and FSH are released, but usually there is more release of LH than FSH. Careful studies of gonadotropin

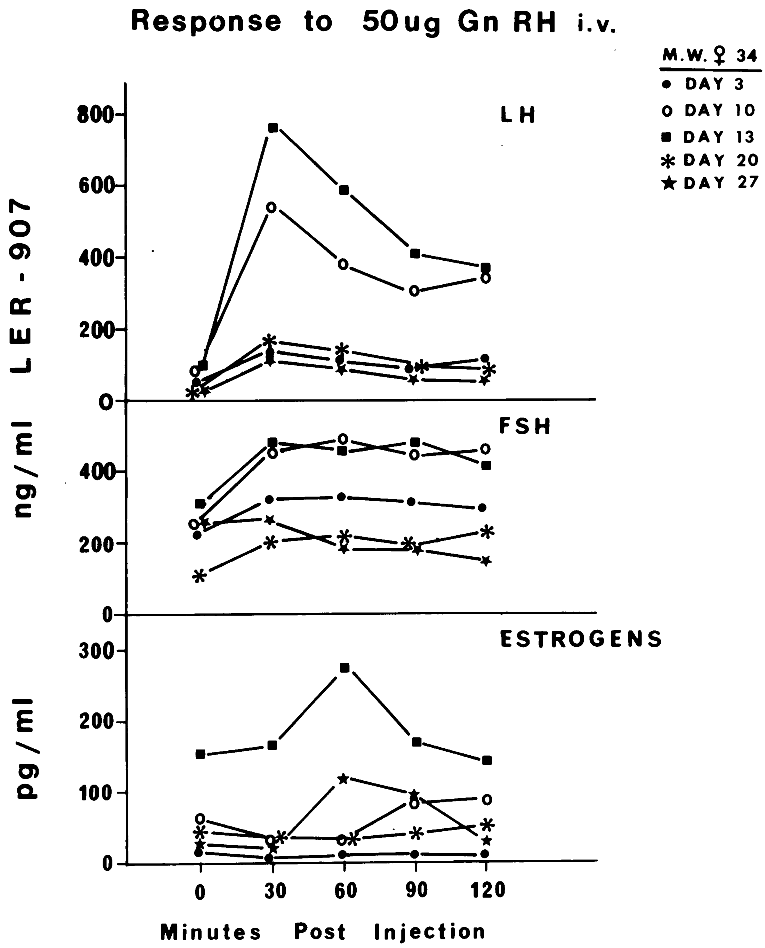



Fig. 3. Effect of 50 μg. GnRH injected intravenously in one subject during different stages of a normal menstrual cycle.

in the normal menstrual cycle reveal that at certain times in the cycle there is divergence between LH and FSH.[10] In the early follicular phase FSH is high and LH is low. At the time of ovulation, there is a very large increase in LH (the "LH surge") and a much smaller increase in FSH. Divergence between LH and FSH are also seen in certain pathological conditions. Recently Bowers isolated from the hypothalamus of the rat a crude extract which had significant FSH-releasing activity.[11] However, each time FSH was released LH was released also, although in smaller amounts. Thus, the question of whether the gonadotropins have one or two releasing hormones has not been settled, but for practical purposes LH-FSH/RH releases both gonadotropins; therefore, many investigators call it gonadotropin-releasing hormone (GnRH). I shall now describe various clinical experiments performed with GnRH. These studies were limited to human beings.

## Dose Response

When increasing quantities of GnRH are injected, there is an increased response in the release of LH and FSH, but above a certain dosage the response remains constant, indicating that there is a maximal level of stimulation by GnRH above which increased dosage levels will not cause more release of LH or FSH.[12] However, interpretation of this type of study is difficult since there is significant individual variability and, in addition, it is difficult to distinguish between a response to GnRH and a physiological pulse of gonadotropins.

## Response to Single Injections in the Normal Menstrual Cycle

When a single dose of GnRH is injected intravenously, there is a prompt release of LH and FSH, which reaches maximal levels for both after 25 to 30 minutes and then gradually declines. The rate of decline is different for LH and FSH. While LH declines quite rapidly and may reach baseline levels after three to four hours, the level of FSH is maintained much longer. This is probably caused by the differing half-lives of LH and FSH.[13] While the half-life of LH is about 19 minutes, the half-life of FSH is about 3.9 hours.[14]

When GnRH was injected at different stages of the menstrual cycle, it was found that the response to a standard dose varied (Figure 3). The response was smallest in the early follicular phase (day three-four), maximal in midcycle (about the time of expected ovulation), and moderate in the midproliferative or midluteal phase. Similar results were found by other investigators.[15, 16] Since in all these experiments the dose of GnRH was the same, the results clearly indicate that the sensitivity of the pituitary gland changes in the course of the cycle. This is probably caused by the changes in the level of gonodal steroids during the cycle. Further evidence substantiating this hypothesis came from amenorrheic hypoestrogenic patients. When GnRH was given to them, the gonadotropic response was minimal. When the same experiment was repeated after pretreatment with estrogens, the increase in

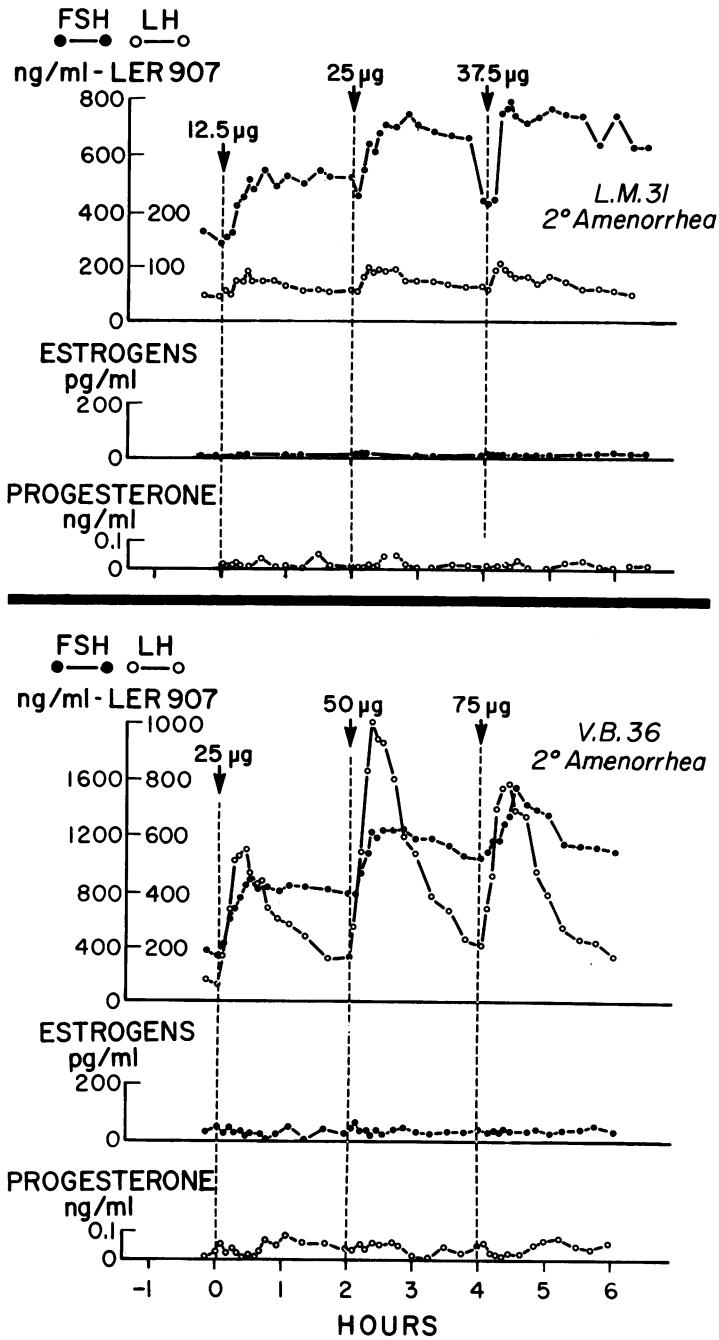



Fig. 4. Levels of LH, FSH, estrogens, and progesterone in plasma after repeated intravenous injections of GnRH in two patients with secondary amenorrhea.

LH and FSH was much higher.[15] This explains further the positive feedback mechanism in the normal cycle. In the early follicular phase the follicles mature under stimulation by a certain level of gonadotropins, but they do not secrete estrogens until a specific stage of development is reached (eighth to ninth day). They then start to produce estrogen, which increases pituitary sensitivity to GnRH. When a follicle reaches maturity the estrogen level is highest. This in turn probably brings the pituitary to its maximal sensitivity to GnRH and a maximal release of gonadotropins (the LH surge), which is believed to trigger ovulation. This mechanism couples follicular maturation with pituitary function. Another mechanism that possibly regulates ovulation may be an increased release of GnRH from the hypothalamus during midcycle. At present it is not clear which mechanism triggers ovulation. It is possible that both mechanisms act simultaneously. GnRH activity has been detected by bioassay in the peripheral blood of women in midcycle at the time of the ovulatory LH surge, but this finding has not yet been confirmed by immunoassay, although immunoassays for GnRH have been developed.[17, 18]

## Responses to GnRH in Abnormal Conditions

Most of our studies were done in abnormal persons: patients with primary and secondary amenorrhea, pituitary tumors, anorexia nervosa, and other conditions. The aim was to differentiate between hypothalamic and pituitary amenorrhea, and if possible to develop a method of testing hypothalamo-pituitary function. Initially the problem looked simple. After injection of GnRH the release of gonadotropins is determined by radioimmunoassay. If adequate release ensues, the pituitary is normal and the cause of amenorrhea is probably in the hypothalamus or central nervous system. If there is no release of gonadotropins, the defect is in the pituitary. However, after we and other investigators[20, 21] had started these studies, we discovered that things are not that simple. When GnRH was injected into patients with idiopathic, secondary amenorrhea, most of them responded normally, indicating that the pituitary was able to release gonadotropins when stimulated. We assumed, therefore, that the disturbance was probably located in the hypothalamus. However, this was a rough approximation, since secondary amenorrhea has many causes. The single-injection test could not pinpoint the anatomical location.

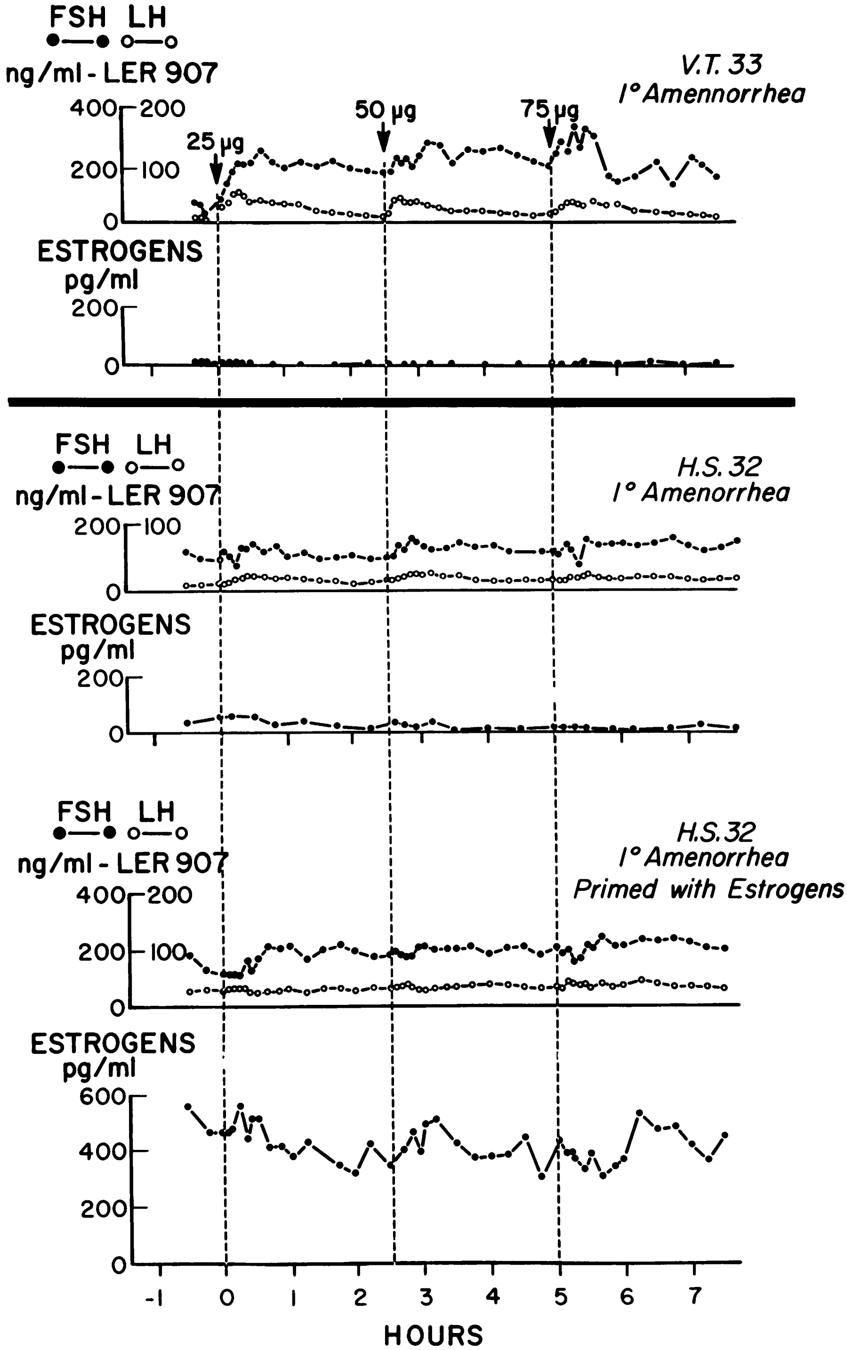



Fig. 5. Levels of LH, FSH, and estrogens in plasma after repeated single injections of GnRH in two patients with primary amenorrhea. *Note:* Patient H. S. was tested twice, once without treatment and the second time after pretreatment with Premarin.

## Repeated Single Injections

After we had given the single intravenous injections to approximately 50 to 60 patients, we recognized that we had not gained much new information. Most of the patients responded—some more and some less, but only a few did not respond at all. Therefore, as a test that could differentiate between hypothalamic and pituitary pathology the procedure was disappointing. Before a practical test could be worked out several basic questions had to be answered:

1) What do we know about the biological reserve of the pituitary and how can it be assessed?

2) Could the pituitary be exhausted, and how?

3) If the pituitary is exhausted, how much time elapses before the gonadotropins are resynthesized and can be released?

Since our questions could not be answered by single-injection experiments, we decided to give repeated injections of GnRH. In the first experiments we gave increasing doses of GnRH to two patients with long-standing secondary amenorrhea and two patients with primary amenorrhea. All but one received a standard series of 25, 50, and then 75 µg. of GnRH every two hours (Figure 4). Blood samples were drawn every five to 15 minutes and assayed for gonadotropins, estrogens, and progesterone. As in the other experiments, there was an almost immediate increase in LH and FSH. Within two hours the level of LH decreased significantly and returned almost to baseline, but that of FSH remained elevated. The first patient (L.M.) had a normal FSH response but very little release of LH. We can speculate that the lack of LH was probably responsible for anovulation and amenorrhea. The second patient (V.B.) responded normally. It should be noted that after each injection the response was similar. There was not much difference in effect between 50 and 75 µg.

In contrast to the clear responses in the two patients with secondary amenorrhea, the response in the two patients with primary amenorrhea was minimal (Figure 5). Patient V.T. had a small rise in FSH and very little in LH, while in patient H.S. the change in both LH and FSH was minimal. In order to ascertain whether estrogen treatment would enhance the responsiveness of the pituitary, the same experiment was repeated on H.S. after she was pretreated for 12 days with conjugated estrogens (Premarin: 1.25 mg./day for three days, 3.5 mg./day for three days, and 5 mg./day for six days) before the GnRH was given.

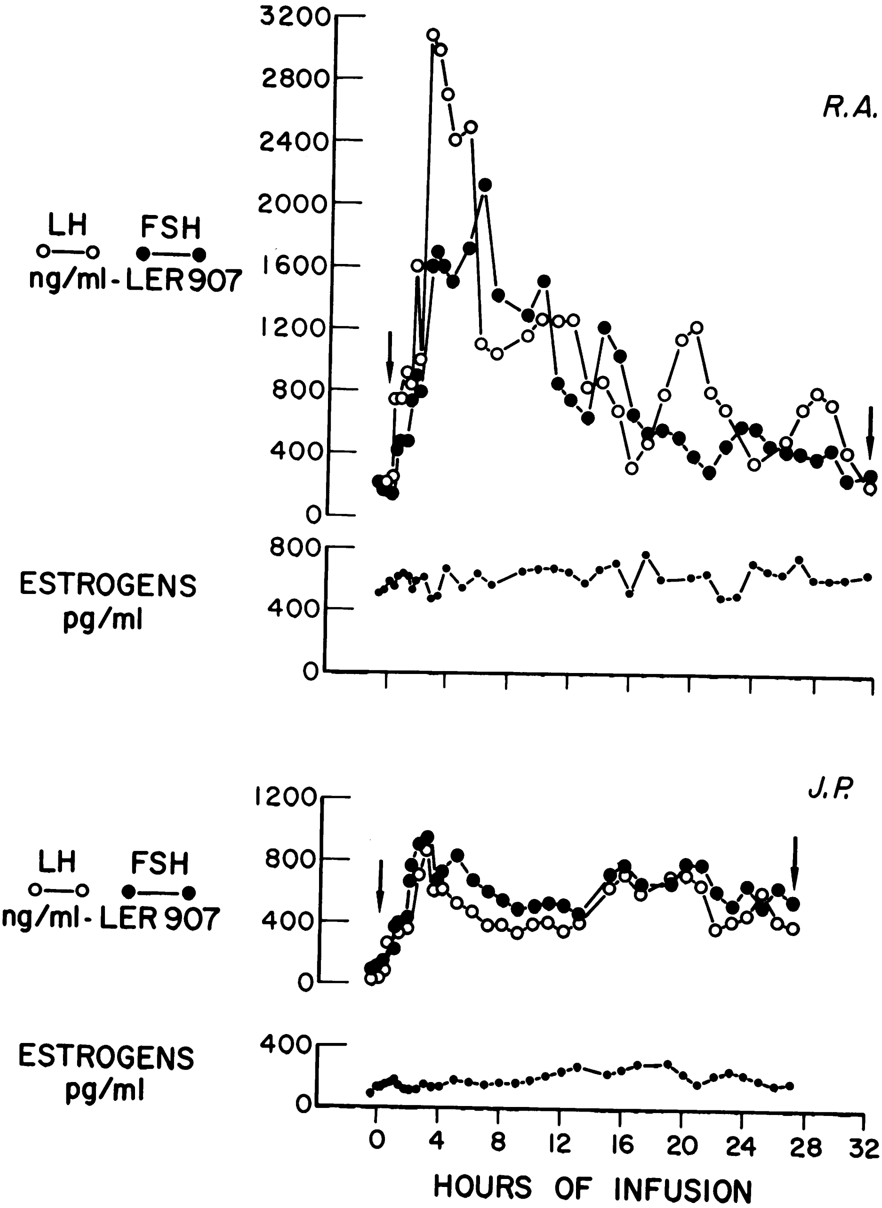



Fig. 6. Levels of LH, FSH, and estrogens in plasma during continuous infusion of GnRH in two normal subjects in the late proliferative phase of the cycle (arrows mark beginning and end of the infusion).

Priming with estrogens did not affect the response. All of these patients were subsequently treated with human menopausal and chorionic gonadotropins (HMG-HCG) and responded with ovulatory cycles. One may conclude from this study that the defect in these patients probably was in the pituitary, which was unable to respond to GnRH.

## Continuous Infusions

Since the single injections gave relatively short-term responses, we decided to give continuous infusions over longer periods of time. These patients were hospitalized. By means of a Harvard pump, a constant infusion of 25 μg./hour of GnRH was given for 22 to 32 hours. First, two normal subjects were given infusions at approximately the late proliferative phase of their cycles (Figure 6). Patient R.A. was so treated on the 13th day of a 28-day cycle. She probably was close to ovulation as indicated by the estrogens—530 pg./ml. There was a significant increase in both gonadotropins. LH reached 3,100 ng./ml. after two hours and FSH reached 2,000 ng./ml. after six hours; then they gradually declined, but nevertheless remained above baseline level as long as the infusion was continued.

Patient J.P. was given the infusion on the 12th day of a 28-day cycle (estrogens 140 pg./ml.). She was not as close to ovulation as the first patient and had a smaller response, but the patterns were similar.

It seems probable that the initial release comprised stored or easily released gonadotropins, while the later level, which was more or less constant, probably represented release of newly synthesized gonadotropins. During this period of time the pituitary could not be exhausted completely. Most striking were the marked oscillations in LH and FSH in both patients throughout the entire period of infusion, despite the constant rate at which GnRH was administered.

## Primary Amenorrhea

Next we gave infusion to four patients who had primary amenorrhea (Figure 7). All were normally developed and had a normal karyotype and low gonadotropins; hence they could be classified as having hypogonadotropic hypogonadism. In all of them the change in LH was minimal; however, two patients had a certain increase in FSH which was less than normal but nevertheless significant. The lack of LH release and the subnormal release of FSH were probably the cause of

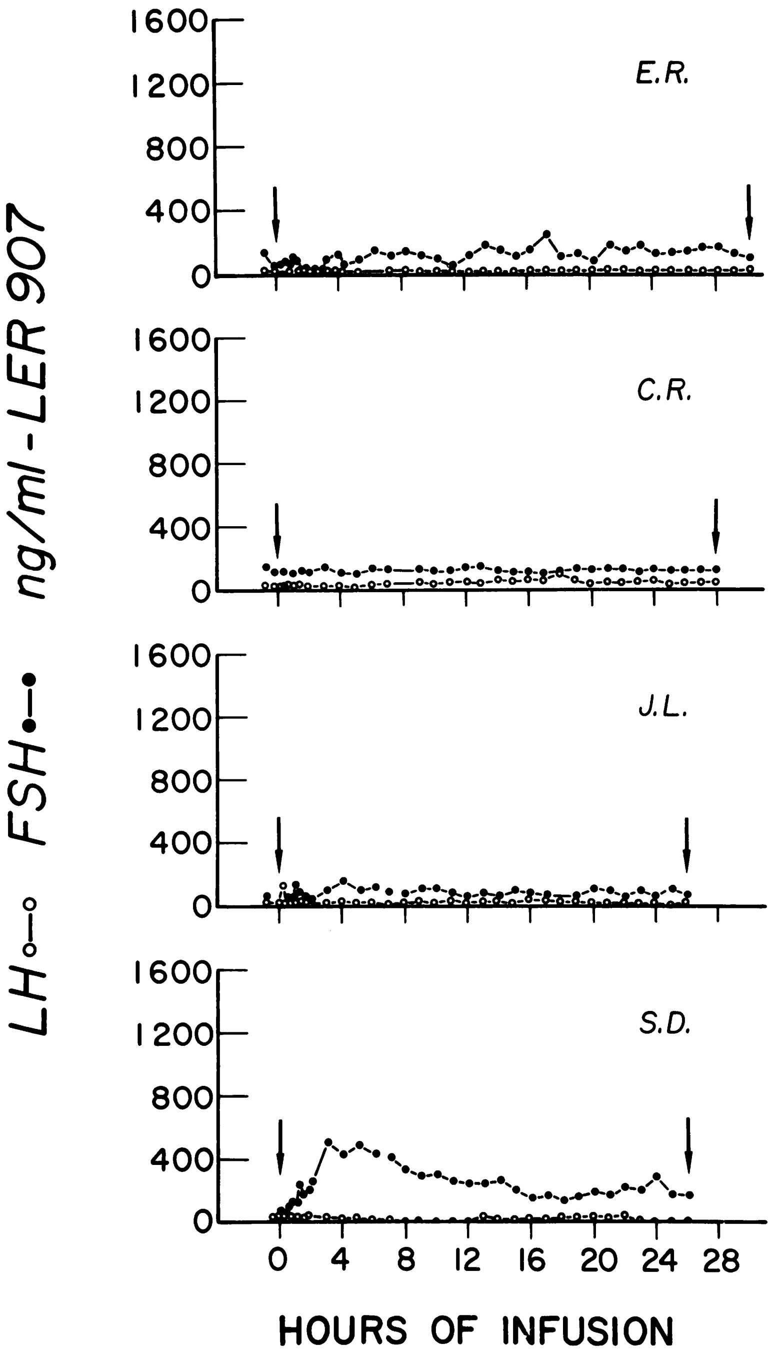



Fig. 7. Levels of LH and FSH in plasma during continuous infusion of GnRH in four patients with primary amenorrhea.

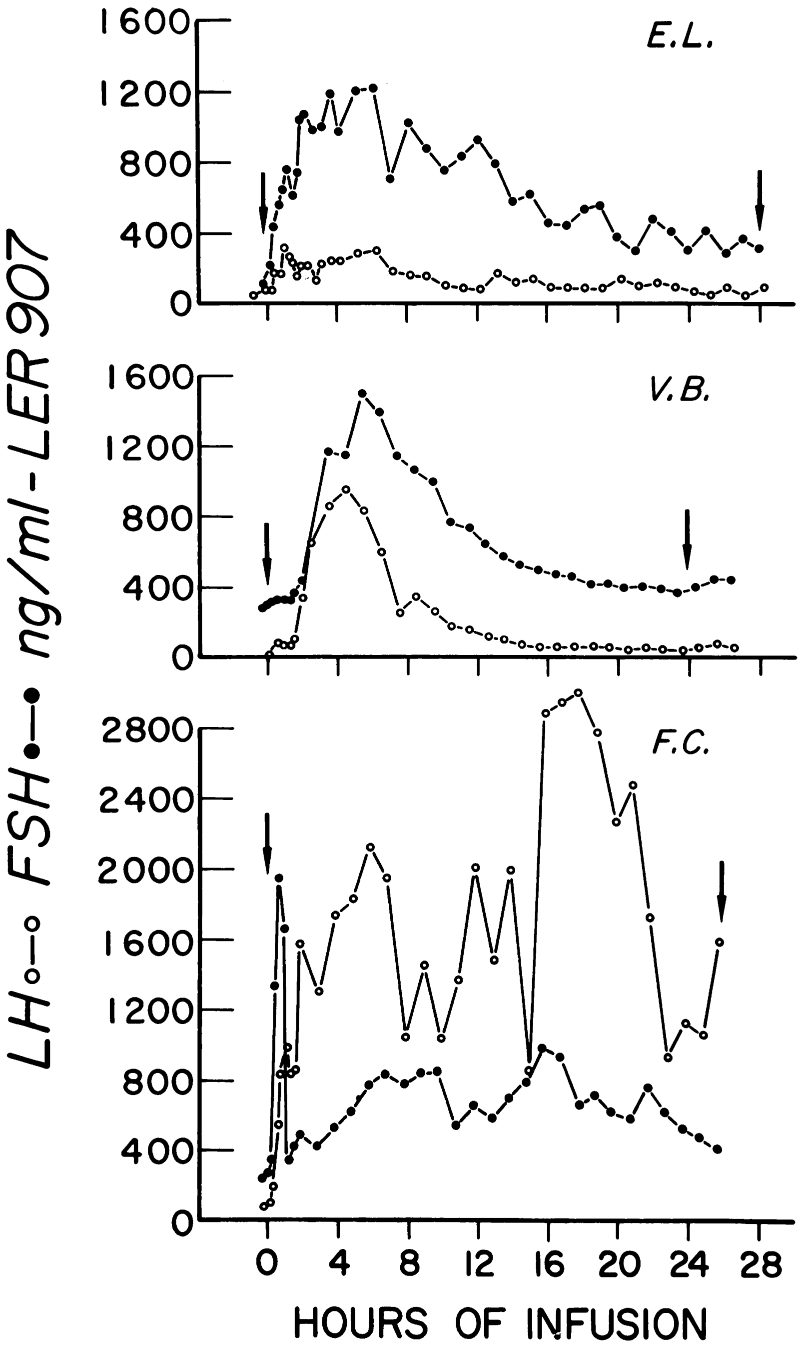



Fig. 8. Levels of LH and FSH in plasma during continuous infusion of GnRH in three patients with secondary idiopathic amenorrhea.

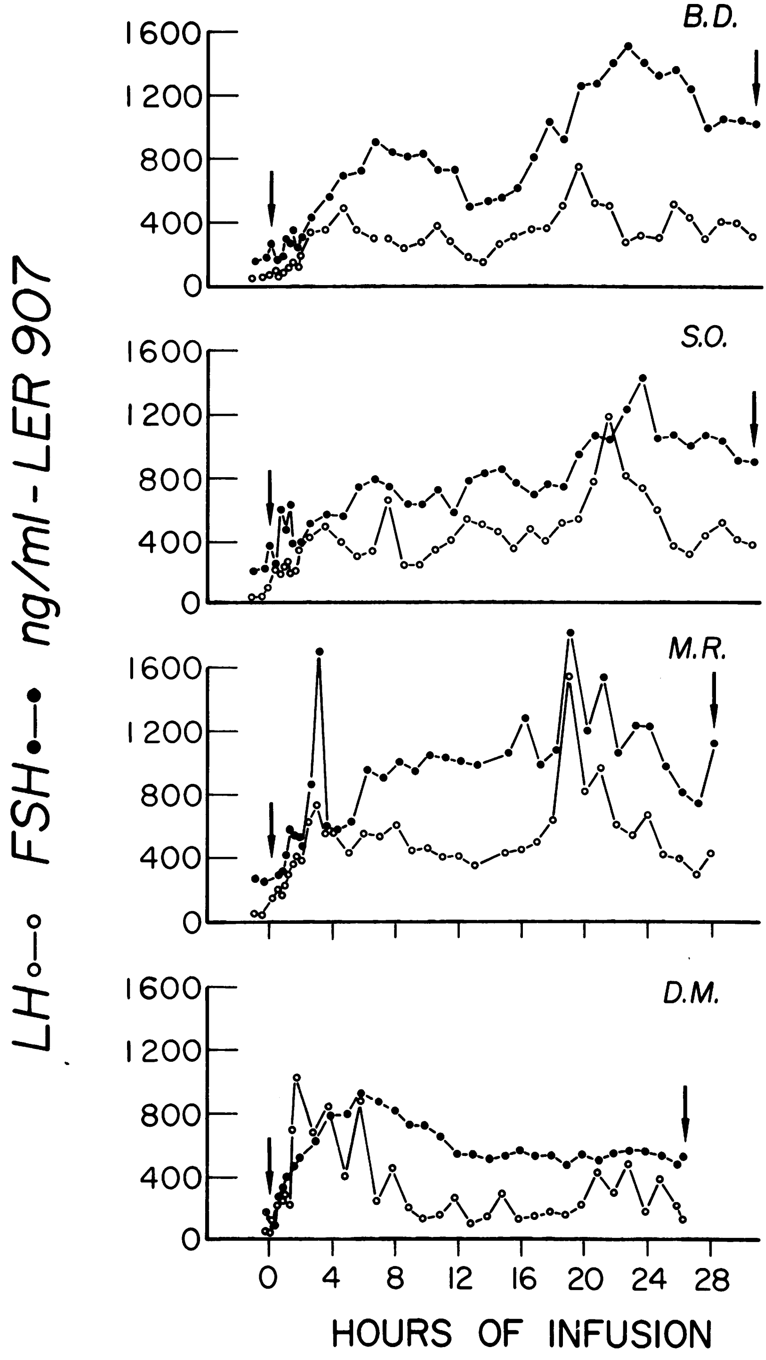



Fig. 9. Levels of LH and FSH in plasma during continuous infusion of GnRH in four patients with secondary amenorrhea following self-imposed loss of weight.

anovulation. However, the ovaries in these patients were normal and were able to respond with ovulatory cycles after treatment with HMG-HCG. One of these patients (S.D.) is now pregnant.

## SECONDARY IDIOPATHIC AMENORRHEA

The next group consisted of three patients with secondary idiopathic amenorrhea (Figure 8). Patients E.L. and V.B. had been treated previously with HMG-HCG for anovulation, and had conceived and delivered live infants. Patient E.L. was given the infusion when her plasma estrogens were 58 pg./ml. A small but significant increase in LH occurred, while FSH rose promptly and to a much higher level. Patient V.B. was of particular interest. She responded normally to single injections of GnRH. Assuming that her functional pituitary reserve was normal, we pretreated this patient with Pergonal; after adequate follicular stimulation was indicated by plasma estrogens of 570 pg./ml., GnRH was infused in an attempt to induce ovulation. In contrast to the results obtained in the normal subjects, there was no response during the first two hours of infusion. After this period the gonadotropins increased, but eight to nine hours later they declined and stayed at baseline levels although the infusion of GnRH was continued for another 15 hours. In the following days there was no significant change in the curve of basal body temperature; the patient bled on the sixth day after the infusion. The level of plasma progesterone never rose above 1 ng./ml., indicating that ovulation was not induced by the GnRH despite adequate follicular stimulation by human menopausal gonadotropins (HMG). In a subsequent cycle similar treatment with HMG-HCG resulted in an ovulatory cycle.

Our conclusion was that this patient's pituitary was unable to release enough gonadotropins to trigger ovulation. This was of particular interest because the single injections indicated a normal response. The fact that a continuous infusion of GnRH caused a limited release of LH and FSH indicated an impairment of pituitary reserve which was not detected during the single injection test. We feel, therefore, that the single injection test may be useful as a screening test for hypothalamo-pituitary function, but that the pituitary reserve can be assessed only by continuous infusion. The optimal dose of GnRH and the duration of the infusion remain to be determined.

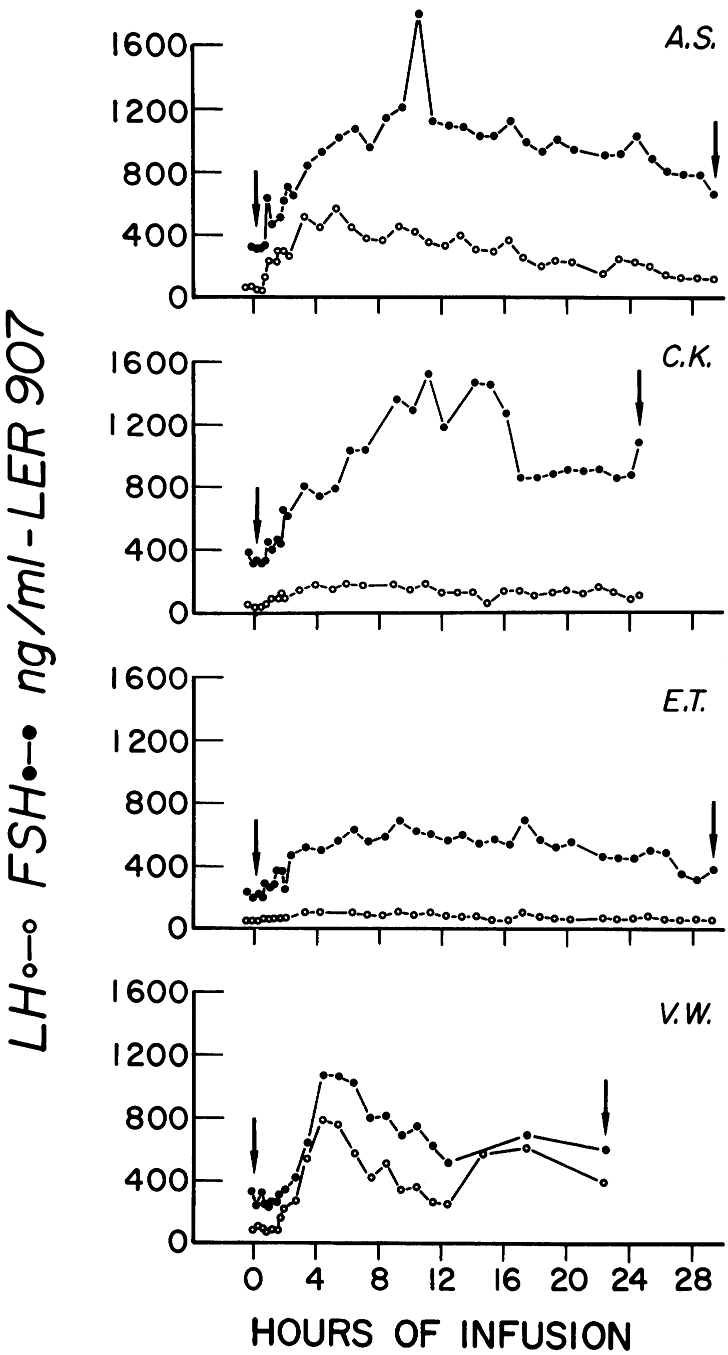



Fig. 10. Levels of LH and FSH in plasma during continuous infusion of GnRH in four patients with pituitary tumors. *Note:* Patient V. W. had empty sella syndrome.

## Amenorrhea Related to Loss of Weight

The four patients with a history of anorexia nervosa (Figure 9) were a heterologous group.They ranged in age from 15 to 28 years. All had lost a large amount of weight within a short period of time during adolescence. Later they regained some of the weight, but remained slim and amenorrheic. All had LH and FSH within normal ranges. The gonadotropin response to GnRH was similar in all four: as in the two normal subjects, there was a clear indication of an early peak in plasma levels of LH and FSH, but in addition there was a later peak, roughly between 18 and 26 hours. This second peak was distinctly more pronounced in these patients, while the initial response seemed to be blunted. The maximal response, reached after 18 to 26 hours, was maintained as long as the infusion was continued. In these patients the pituitary appears to have a decreased sensitivity to GnRH and characteristically requires an abnormally long stimulus, but the ability to respond is probably normal. Whether this decreased sensitivity to GnRH is caused by the long absence of estrogens or other factors is not known.

## Amenorrhea Related to Pituitary Tumor

Three patients with pituitary tumors and one patient with the "empty sella syndrome" were included in the study (Figure 10). All three tumor patients presented with secondary amenorrhea and galactorrhea. After the study was carried out, patient C.K. underwent an operation, which confirmed the presence of a pituitary tumor. In patient A.S. a tumor was indicated by an enlarged sella turcica and a plasma prolactin level above 200 ng./ml., but there were no neurological or visual disturbances. Patient E. T. had previously received radiation therapy to the pituitary. It is interesting to note that in these three patients the infusions of GnRH produced a marked rise in FSH levels, although this occurred after considerable delay: a maximum was not reached until the ninth to 11th hour of infusion. In distinct contrast, the LH response was subnormal in A.S., negligible in patient C.K., and absent in E.T., the irradiated patient. This pattern of response is very different from that observed in patient F. C. (Figure 8), a patient with secondary amenorrhea and galactorrhea without evidence of a pituitary tumor, in whom LH was by far the overriding gonadotropin and the response to GnRH was most marked. The responses of these patients seem to point to selective unresponsiveness of LH to GnRH, which would suggest that the mechanism involved in the production of LH is more sensitive to insult and more vulnerable than that responsible for FSH. Patient V. W. had secondary amenorrhea and an enlarged sella turica, but after extensive clinical study she was found to be endocrinologically normal and was believed to have empty sella syndrome.

## Integrated Pituitary Tests.

Several investigators have suggested combining pituitary-stimulating substances into a group of "integrated pituitary tests" to assess pituitary function: hypoglycemia for GH response, thyrotropin-releasing hormone (TRH) for thyroid-stimulating hormone (TSH), and prolactin and GnRH for LH and FSH.[21] These tests can be performed simultaneously, and it is possible that in the future we may have a battery of tests for pituitary function analogous to what we now have for liver function. Though it appears promising, our experience with this method is too limited to draw early conclusions.

## Induction of Follicular Maturation and Ovulation with GnRH

In 1971 Kastin reported the first case of successful induction of ovulation and pregnancy with porcine GnRH.[22] However, careful examination of his report revealed that his case was not exactly one of hypogonadotrophic infertility. Since then several additional reports of successful induction of ovulation and pregnancy have been published.[23, 24, 25] In all these cases the patients belonged to a heterologous group; some had low, others had normal gonadotropins, many had polycystic ovaries. The dose of GnRH varied and there was much individual variation in gonadal response to stimulation by GnRH. All these studies lacked long-term follow-up of the biochemical changes induced by GnRH. Our aim is to induce a sustained gonadotrophic release which will induce follicular maturation, as we can now do with human menopausal gonadotropins (Pergonal), and then to trigger ovulation, as we do with human chorionic gonadotropins. So far, sustained release of gonadotropin has not been achieved with GnRH. After a single GnRH injection there is an increase in LH and FSH; this is maintained for several hours only and then returns to the baseline. Whether this temporary increase in gonadotropins is sufficient to stimulate follicular growth and development in all cases is not clear.

We have tried to induce ovulation in two patients with long-standing secondary amenorrhea who had gonadotropins in the lower range of normal levels. They were given 150 $\mu$g. GnRH per day by subcutaneous injection for 17 to 18 days and daily blood samples were assayed for LH, FSH, and estrogens. Only temporary increases in LH and FSH were noted and there was no evidence of follicular maturation. Both patients responded well to Pergonal and one conceived. We concluded that, at least at the given dose and mode of administration, GnRH did not release enough gonadotropins for long enough to cause follicular maturation and ovulation. Dr. Carl Gemzel has had similar results.[26]

It is too early to say whether GnRH will be useful clinically for induction of ovulation. Perhaps different doses and different regimens and routes of administration are needed: e.g., the use of long-acting analogs. Another method is suggested by a recent report of intranasal administration of GnRH that resulted in significant increases in LH and FSH which were not different from the response to intravenous injections.[27] If GnRH is sniffed every few hours the gonadotropins may be kept elevated for prolonged periods of time, causing follicular maturation. Within the next year or so we shall be learning much more about this method.

## Possible Control of Conception

Since GnRH is a relatively simple molecule, chemical manipulations are possible to prevent conception and several analogs have already been developed.[28] Further research on analogs might go in two directions: 1) long-acting analogs with physiological activity similar to that of GnRH and 2) analogs that compete with GnRH and disrupt the normal cycle. Both groups of substances may be ideal contraceptives, since they act locally in the hypothalmus or pituitary and interrupt the cycle without having the general metabolic effects of the oral contraceptives. Another possibility for preventing conception is immunization against GnRH. Antiserums to GnRH have been produced recently in laboratory animals,[29] but clinical studies in humans are not yet available. These, of course, are speculations and we do not know how they will develop. However, preliminary results of experiments done by Schally and others[30] are encouraging.

## Summary

We have reviewed briefly the historical background of the releasing factors and the anatomy of the hypothalamo-pituitary region. Several clinical studies with GnRH given in single injections, repeated single injections, and infusions, were described. Finally, the induction of ovulation with GnRH and the possibilities of using analogs of GnRH for contraception were discussed.

## *REFERENCES*

1. Harris, G. W.: Induction of ovulation in rabbit by electrical stimulation of hypothalamohypophyseal mechanism. *Proc. Roy, Soc.* [Biol.] *122*:374, 1937.
2. Scharrer, E. and Scharrer, B.: Hormones produced by neurosecretory cells. *Recent Progr. Hormone Res. 10*:183, 1954.
3. Harris, G. W.: *Neural Control of the Pituitary Gland.* London, Arnold, 1955.
4. Wislocki, G. B. and King, L. S.: The permeability of the hypophysis and hypothalamus to vital dyes, with a study of the hypophyseal vascular supply. *Amer. J. Anat. 58*:421, 1936.
5. Schally, A. V., Arimura, A., and Kastin, A. J.: Hypothalamic regulatory hormones. *Science 179*:341, 1973.
6. Davidson, J. M.: Feedback Control of Gonadotropin Secretion. In: *Frontiers in Neuroendocrinology,* Ganong, W. F. and Martini, L., editors. New York, Oxford University Press, 1969, p. 343.
7. Schally, A. V., Arimura, A., Kastin, A. J., Matsuo, H., Baba, Y., Redding, T. W., Nair, R. M. G., Debeljuk, L., and White, W. F.: Gonadotropin-releasing hormone: One polypeptide regulates secretion of luteinizing and follicular stimulating hormones. *Science 173*:1036, 1971.
8. Schally, A. V., Arimura, A., Baba, Y., Nair, R. M. G., Matsuo, H., Redding, T. W., Debeljuk, L., and White, W. F.: Isolation and properties of the FSH and LH-releasing hormone. *Biochem. Biophys. Res. Commun. 43*:393, 1971.
9. Schally, A. V., Kastin, A. J., and Arimura, A.: The hypothalamus and reproduction. *Amer. J. Obstet. Gynec. 114*: 423, 1972.
10. Speroff, L. and Vande Wiele, R. L.: Regulation of the human menstrual cycle. *Amer. J. Obstet. Gynec. 109*:234, 1971.
11. Bowers, C. Y., Currie, B. L., Johansson, N. G., and Folkers, K.: Biological evidence that separate hypothalamic hormones release the follicle stimulating and luteinizing hormones. *Biochem. Biophys. Res. Commun. 50*:20, 1973.
12. Reber, R., Yen, S. S. C., Vandenberg, G., Naftolin, E., Ehara, Y., Engblom, S., Ryan, K. J., Rivier, J., Amos, M., and Guillamin, R.: Gonadotropin response to synthetic LRF: Dose-response relationship in men. *J. Clin. Endocr. 36*: 10, 1973.
13. Gay, V. L., Midley, A. R., Jr., and Niswender, G. D.: Pattern of gonadotropin secretion associated with ovulation. *Fed. Proc. 29*:1880, 1970.
14. Yen, S. S. C., Llerena, L. A., Pearson, O. H., and Little, A. S.: Disappearance rates of endogenous follicle stimulating hormone in serum following surgical hypophysectomy in men. *J. Clin. Endocr. 30*:325, 1970.
15. Nillius, S. J. and Wide, L.: Variation in LH and FSH response to LH releasing hormone. *J. Obstet. Gynaec. Brit. Comm. 79*:865, 1972.
16. Yen, S. S. C., Vandenberg, G., Reber, R., and Ehara, Y.: Variations of pituitary responsiveness to synthetic LRF during different phases of the menstrual cycle. *J. Clin. Endocr. 35*:931, 1972.
17. Neff, T. M., Akbar, A. M., Hopwood,

M. L., and Niswander, G. D.: Gonadotropin-releasing hormone levels in serum of sheep as measured by radioimmunoassay. *Fifty-fifth Ann. Meeting Endocrin Soc.*, Chicago, June 20-22, 1973.

18. Keye, W. R., Kelch, R. P., Niswender, G. D., and Jaffe, R. B.: Quantitation of endogenous and exogenous gonadotropin-releasing hormone by radio-immunoassay. *J. Clin. Endocr. 36*:1263, 1973.
19. Malacara, J. N., Segler, L. E., and Reichlin, S.: Luteinizing hormone releasing factor activity in peripheral blood from women during the midcycle luteinizing hormone ovulatory surge. *J. Clin. Endocr. 34*:271, 1972.
20. Nillius, S. J. and Wide, L.: The LH releasing hormone test in 31 women with secondary amenorrhea. *J. Obstet. Gynaec. Brit. Comm. 97*:874, 1972.
21. Rakoff, J. S., Ehara, Y., Siler, T. M., and Yen, S. S. C.: A six hour direct functional test of the anterior pituitary gland. *Twentieth Ann. Meeting Soc. Gynecological Invest.* Atlanta, Ga., March 28-30, 1973.
22. Kastin, A. J., Zarate, A., Midgley, A. R., Canales, E. S., and Schally, A. V.: Ovulation confirmed by pregnancy after infusion of poraine LH-RH. *J. Clin. Endocr. 33*:980, 1971.
23. Kaller, P. L.: Treatment of anovulation with synthetic luteinizing hormone-releasing hormone. *Amer. J. Obstet. Gynec. 116*:698, 1973.
24. Zarate, A., Canales, E. S., Sonia, J., Gonzales, A., Schally, A. V., and Kastin, A. J.: Further observations on the therapy of anovulatory infertility with synthetic luteinizing hormone-releasing hormone. *Fertil. Steril. 25*:3, 1974.
25. Zanartu, J., Dabanceus, A., Kastin, A. J., and Schally, A. V.: Effect of synthetic hypothalamic gonadotropin-releasing hormone (FSH/LH-RH) in anovulatory sterility. *Fertil. Steril. 25*: 160, 1974.
26. Gemzel, C.: Personal communication.
27. London, D. R., Butt, W. R., Lynch, S. S., Marshal, J. C., Owush, S., Robinson, W. R., and Stephenson, J. M.: Hormonal response to intranasal luteinizing hormone-releasing hormone. *J. Clin. Endocr. 37*:829, 1973.
28. Schally, A. V., Arimura, A., Carter, W. M., Redding, T. W., Geigar, R., Konig, W., Wissman, H., Jaeger, G., Sandow, J., Yanaihara, N., Yanaihara, C., Moshimoto, T., and Sakagami, M.: Luteinizing hormone-releasing hormone (LH-RH) activity of some synthetic polypeptides. 1. Fragments shorter than decapeptides. *Biochem. Biophys. Res. Commun. 48*:366, 1972.
29. Jutisz, M. and Kerdelhue, B.: In-vitro Studies on Synthetic LH-RH and its Assay in Plasma Using a Radioimmunoassay Method. In: *Hypothalamic Hypophysiotropic Hormones—Physiological and Clinical Studies,* Gual, C. and Rosenberg, E., editors. Amsterdam, Excerpta Medica, 1973, p. 98.
30. Schally, A. V. Personal communication.

## *ERRATA*

The following corrections apply to the book review in the September 1974 issue of the *Bulletin:* Pages 955-58: for Donald Eaton read Dorman B. Eaton. Page 960: John Howard, High Sheriff of Bedfordshire, was required to inspect prisons, not hospitals or lazarettos.